

RESEARCH ARTICLE

ELISA-based quantification of neurotrophic growth factors in urine from prostate cancer patients

Brayden March^{1,2,3,4} | Kathleen Rebecca Lockhart⁴ | Sam Faulkner^{1,2}  | Markus Smolny⁵ | Robert Rush^{5,6} | Hubert Hondermarck^{1,2}

¹School of Biomedical Sciences & Pharmacy, The University of Newcastle, Callaghan, NSW, Australia

²Hunter Medical Research Institute, New Lambton, NSW, Australia

³School of Medicine & Public Health, The University of Newcastle, Callaghan, NSW, Australia

⁴Department of Urology, John Hunter Hospital, New Lambton Heights, NSW, Australia

⁵Biosensis Pty Ltd, Thebarton, SA, Australia

⁶Department of Human Physiology, Flinders University, Adelaide, SA, Australia

Correspondence

Hubert Hondermarck, School of Biomedical Sciences and Pharmacy, Life Sciences Building (LS3-35), University of Newcastle, Callaghan, NSW 2308, Australia.

Email: hubert.hondermarck@newcastle.edu.au

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Abstract

Non-invasive procedures are needed for prostate cancer management, and urine represents a potential source of new biomarkers with translational value. Recent evidence has shown that the growth of new nerves in the tumor microenvironment is essential to prostate cancer progression. Neurotrophic growth factors are expressed by prostate cancer cells and contribute to prostate tumor innervation, but their presence in urine is unclear. In the present study, we have assayed the concentration of neurotrophic factors in the urine of prostate cancer patients. Urine was collected from a prospective cohort of 45 men with prostate cancer versus 30 men without cancer and enzyme-linked immunosorbent assay was used to quantify nerve growth factor (NGF) and its precursor proNGF, brain-derived neurotrophic factor (BDNF) and proBDNF, neurotrophin-3, neurotrophin-4/5, and glia-derived neurotrophic growth factor. The results show that neurotrophic factors are detectable in various concentrations in both cancer and healthy urine, but no significant difference was found. Also, no association was observed between neurotrophic factor concentrations and prostate cancer grade. This study is the first quantification of neurotrophins in urine, and although no significant differences were observed between prostate cancer patients versus those without prostate cancer, or between prostate cancers of various grades, the potential value of neurotrophins for prostate cancer diagnosis and prognosis warrants further investigations in larger patient cohorts.

KEYWORDS

biomarkers, diagnosis, neurotrophic growth factors, prognosis, prostate cancer, urine

1 | INTRODUCTION

Prostate cancer is the second most common cancer and the fifth leading cause of cancer-related death in men worldwide, and its incidence is projected to increase over the coming decades.^{1,2} Prostate-specific antigen (PSA) was introduced over 30 years ago³ and remains the first-line and most commonly used serum biomarker for prostate cancer detection.⁴ Although PSA has improved the diagnosis of early stage and localized prostate cancer, controversy remains over its suitability as a screening tool in current clinical practice. Large, multi-center randomized trials have shown conflicting results in the ability of PSA screening to reduce prostate cancer mortality.⁵⁻⁷ In addition, there is significant harm associated with population-based PSA screening, including false-positive results⁸ and overdiagnosis of clinically indolent cancer which would have never caused symptoms in the patient's lifetime.⁹⁻¹¹ Therefore, novel biomarkers are required for screening and risk stratification of prostate cancer.

The development of new nerves in the tumor microenvironment is an emerging hallmark of cancer.^{12,13} In prostate cancer, the denervation of sympathetic and parasympathetic nerves strongly reduces tumor progression and the formation of metastases.¹⁴ Sympathetic and parasympathetic nerves activate adrenergic and cholinergic signaling in prostate tumor cells, which results in the stimulation of cancer cell proliferation and dissemination.¹⁴ In addition, sympathetic nerves can also induce an angio-metabolic switch, through the release of noradrenaline, resulting in the vascularization of prostate tumors, thus further promoting tumor growth and dissemination.¹⁵ New therapeutic strategies aiming to target neurosignaling in prostate cancer are now emerging.¹⁶ The cause of nerves infiltrating the tumor microenvironment of prostate cancer remains to be fully elucidated but the production of neurotrophic growth factors by cancer cells is a clear driver of prostate cancer innervation. The precursor for nerve growth factor (proNGF) in particular has been shown to be associated with nerve infiltration and tumor grade in prostate cancer.¹⁷ However, other neurotrophic factors are also expressed in prostate cancer¹⁸ and they may also participate in tumor innervation.

Neurotrophic factors of the neurotrophin family, which are structurally and functionally related to nerve growth factor (NGF), are of interest as drivers of prostate cancer innervation^{16,17} and biomarkers of the disease.¹⁹ In humans, there are four neurotrophins: NGF, brain-derived-neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). Neurotrophins are synthesized as precursor forms, proneurotrophins, which are subsequently processed into mature neurotrophins; however, both proneurotrophins and neurotrophins can bind to the same cell surface receptors. Although neurotrophins are mainly described for their involvement in the development of the nervous

system, intriguingly the prostate is a rich source of neurotrophins.²⁰ Alterations in neurotrophin receptor expression, and the acquisition of autocrine and paracrine neurotrophin stimulation occur during prostate carcinogenesis.¹⁸ Immunohistochemistry studies have demonstrated a positive correlation between the expression of NGF, proNGF, BDNF, and prostate cancer aggressiveness, as reflected with tumor grade and stage.^{17,20-22} Aside from neurotrophins, glia-derived neurotrophic growth factor (GDNF) is also described in prostate cancer. GDNF stimulates the invasiveness of prostate cancer cells²³ and the invasion of nerves by cancer cells, a process called perineural invasion,²⁴ through the activation of its tyrosine kinase receptor RET. Therefore, given their involvement in prostate cancer, neurotrophins and GDNF may have potential as clinical biomarkers.

Urine has been proposed as a readily available substrate for prostatic biomarkers.²⁵ The urethra carries urine away from the bladder to be expelled, passing through the prostate whereby it merges with the ejaculatory ducts from which prostatic fluid is propelled into the urethra. The passage of urine through the prostate may allow for the early diagnosis of prostate cancer through the isolation of tumor cells and their components in urine sediment.²⁵ Interestingly, NGF has already been reported to be present in human urine and suggested as a possible biomarker of tumor aggressiveness.¹⁹ As other neurotrophic factors are also produced by prostate cancer cells,^{17,18,20} it can be hypothesized that they also could be detected in urine and be useful as clinical biomarkers for prostate cancer.

In this study, we have used enzyme-linked immunosorbent assay (ELISA) to detect and quantify neurotrophic growth factors in the urine of prostate cancer patients from a prospective cohort of men presenting for investigation of suspected prostate cancer. The results show that neurotrophic factors can be detected at various levels in human urine. Although we have not evidenced any significant differences in the level of neurotrophic factors between cancer versus non-cancer patients, or between patients bearing tumors of different grades, the data call for more investigations into the potential diagnostic or prognostic value of neurotrophic factors for the management of prostate cancer.

2 | MATERIALS AND METHODS

The workflow of the study is presented in Figure 1.

2.1 | Study population

The Human Research and Ethics Committee of the Hunter New England Local Health District approved the research for this study (Reference No. 18/03/21/4.09). We prospectively

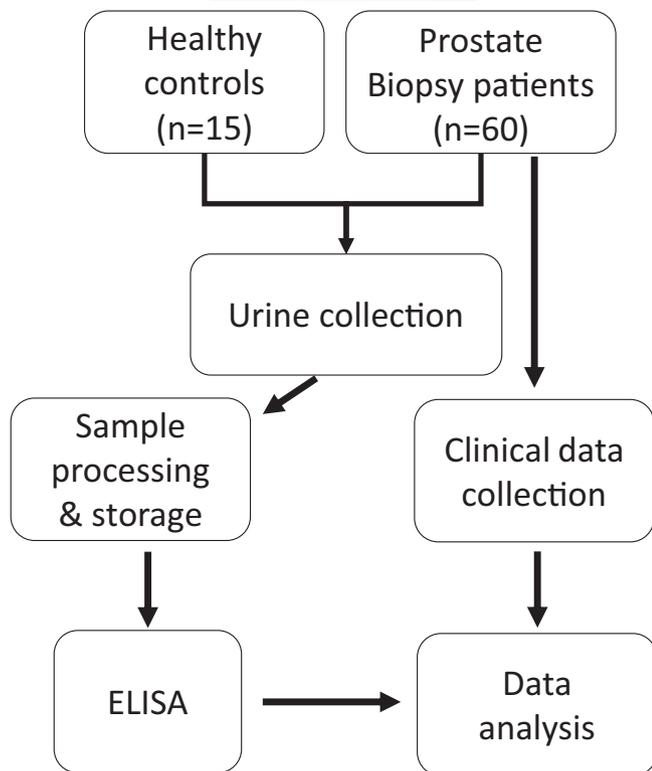


FIGURE 1 Study workflow. Urine samples were prospectively collected from patients undergoing biopsy for prostate cancer and from control healthy men of the same age group. After pathological analysis, some biopsied men turned out not to have prostate cancer ($n = 15$) and were included in the non-cancer group ($n = 30$), as opposed to patients with confirmed prostate cancer ($n = 45$). Urine samples were processed as described in Material and methods and neurotrophic factors were assayed by ELISA. Neurotrophic factor concentrations were then statistically compared to clinicopathological parameters

recruited patients presenting for a trans-rectal ultrasound guided prostate biopsy at Belmont or Maitland Hospitals, New South Wales, Australia. Written consent forms were obtained in all cases. Patients were excluded if they had a personal history of cancer within the previous 10 years. In addition, healthy controls were volunteers recruited through the Hunter Medical Research Institute Research register. Control subjects were more than 45 years of age, did not have a personal or family history of prostate cancer, and did not have any significant lower urinary tract symptoms. A total of 75 clinical samples were collected, with 60 participants recruited in the context of a prostate biopsy, and 15 participants recruited as healthy controls. Of note, 15 of the 60 biopsied men turned out not to have prostate cancer and were therefore classified as non-cancer, making the total number of non-cancer patients equal to 30 versus 45 cancer patients. Mean age of participants was 65 (range: 28–79). Patient demographics are displayed in Table 1. Of the prostate biopsy group, the indication for biopsy was active surveillance of low-grade prostate cancer in 18 men (these men underwent repeat biopsy), and investigation of elevated PSA in 42 cases

TABLE 1 Patient characteristics

Neurotrophin	<i>n</i>	%
Age (range)	68 (28–79)	
Controls	15	20
Biopsy	45	80
Biopsy characteristics		
Negative	15	20
Positive	45	60
ISUP1-2	29	38.6
ISUP3-5	16	21.3
PNI	17	22.6
Mean PSA	9.8 (1.2–61)	
Negative	5.9 (1.2–9.8)	
ISUP1-2	6.8 (1.1–12.5)	
ISUP3-5	18.3 (5.8–61)	

(initial biopsy). Prostate biopsy was performed via transrectal ultrasound-guided needle biopsy and reported by the anatomical pathology service of John Hunter Hospital, Newcastle. Clinicopathological data were collected from the histopathology report of the prostate biopsies. Parameters collected included preoperative PSA, number of cores taken, number of cores positive for cancer, and overall ISUP Grade Group.

2.2 | Urine sample collection and processing

Men were asked to provide a first-pass urine sample when they had a desire to void. Samples were immediately tested with standard urine analysis strips (Siemens Multistix 10, SG), then put on ice and transferred to the laboratory to be processed within 4 h of collection. All samples were processed as per the Human Kidney & Urine Proteome Project protocol.²³ Briefly, samples were centrifuged for 10 min at 1000 g at 4°C, aliquoted into 1.5 ml units, and stored at –80°C. Creatinine was measured using a urine-specific colorimetric assay (Cayman Chemicals, No. 500701).

2.3 | ELISA

Urinary concentration of neurotrophic growth factors was determined using an ELISA system developed by Biosensis Pty Ltd. All mature neurotrophins (NGF, BDNF, NT3, and NT4/5), two pro-neurotrophins (proNGF and proBDNF), and GDNF were assayed. Biosensis ELISA Kit product numbers were as follows: BEK-2212 (NGF), BEK-2226 (proNGF), BEK-2211 (BDNF), BEK-2237 (proBDNF), BEK-2221 (NT-3), BEK-2218 (NT-4/5), and BEK-2222 (GDNF). These sandwich assays allow quantification of the mature or pro-neurotrophin

using a sensitive and specific pre-coated plate with capture antibody, a biotinylated detection antibody and horseradish peroxidase-conjugated streptavidin. All kits were tested as per the manufacturer's instructions. Although all controls have been performed by the manufacturers, standard curve validation, spike recovery, linearity of dilution, intraplate reproducibility, limit of detection, and limit of quantitation were independently validated in our laboratory. All samples were tested non-diluted. All plates were read with a SPECTRAMax plate reader (Molecular Devices LLC, M3). Optimization of the TMB incubation step was performed with every ELISA experiment. Plates were incubated in a box, and absorbance at 650 nm first read at the minimum incubation time specified in the kit protocol, and then every 5 min thereafter. TMB stop solution was added when the absorbance value (650 nm) of the highest concentration of the standard curve either reached 1.0 or above, or plateaued, and the rate of increase in absorbance was less than 0.3 every 5 min.

2.4 | Statistical analysis

Student's *t*-test was used to evaluate the mean difference in urinary neurotrophins between biopsy cancer positive cases (cancer patients), and healthy controls with biopsy negative cases together (non-cancer subjects), and between low-grade cancers (ISUP1-2) and high-grade cancers (ISUP3-5). Both raw neurotrophin measurements and creatinine-adjusted values were evaluated. GraphPad Prism version 8.43 for Windows was used for all statistical analysis.

3 | RESULTS

NGF and proNGF, BDNF and proBDNF, NT-3, NT4-5, and GDNF were assayed by ELISA in urine samples from prostate cancer patients versus non-cancer men and the results are presented in Table 2 and Figures 2–6.

3.1 | Quantification of NGF and proNGF in urine

NGF and proNGF concentrations in urine samples from cancer patients versus non-cancer patients are presented in Table 2 and Figure 2.

NGF was detected in 21/45 (46%) of urine samples from cancer patients and only in 9/30 (30%) of urine samples from non-cancer subjects (Table 2), and the mean NGF concentration was 6.5 pg/ml (95% CI, 2.7–10) in cancer urine samples versus 5.4 pg/ml (95% CI, 0.91–9.9) in non-cancer urine samples. When considering prostate cancer grades, 11/29 (34%) of ISUP1-2 urine samples and 10/16 (62%) of ISUP3-5 were positive for NGF. The mean NGF concentration was 4.9 pg/ml (95% CI, 0.91–8.9) in ISUP1-2 versus 8.9 pg/ml (95% CI, 0.96–17) in ISUP3-5. There was no significant difference in NGF concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 2A). Normalization of NGF concentration to creatinine exhibited the same distribution with no significant difference between groups (Figure 2B).

ProNGF was detected in 24/45 (53%) of urine samples from cancer patients and 20/30 (66%) of urine samples from non-cancer subjects (Table 2), and the mean proNGF concentration was 770 pg/ml (95% CI, 500–1000) in cancer urine samples versus 880 pg/ml (95% CI, 550–1200) in non-cancer urine samples. When considering prostate cancer grades, 14/29 (48%) of ISUP1-2 urine samples and 10/16 (62%) of ISUP3-5 were positive for proNGF. The mean proNGF concentration was 670 pg/ml (95% CI, 350–1000) in ISUP1-2 versus 940 pg/ml (95% CI, 460–1400) in ISUP3-5. There was no significant difference in proNGF concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 2C). Normalization of proNGF concentration to creatinine exhibited the same distribution with no significant differences between groups (Figure 2D).

TABLE 2 Descriptive statistics of urinary concentrations of neurotrophic factors. All measurements are indicated in pg/ml. Positive = number of positive cases/total cases

Neurotrophin (pg/ml)	Non-cancer patients		Cancer patients		ISUP 1-2		ISUP 3-5	
	Positive	Mean concentration (95% CI)	Positive	Mean concentration (95% CI)	Positive	Mean concentration (95% CI)	Positive	Mean concentration (95% CI)
NGF	9/30	5.4 (0.91–9.9)	21/45	6.5 (2.7–10)	11/29	4.9 (0.91–8.9)	10/16	8.9 (0.96–17)
proNGF	20/30	880 (550–1200)	24/45	770 (500–1000)	14/29	670 (350–1000)	10/16	940 (460–1400)
BDNF	15/26	4.2 (1.9–6.5)	23/36	4.32 (2.7–5.9)	12/23	3.4 (1.4–5.4)	1/12	6.4 (3.4–9.5)
proBDNF	18/30	34 (13–55)	30/45	38 (22–53)	16/29	29 (13–45)	14/16	54 (21–87)
NT3	14/30	32 (16–47)	15/45	27.8 (14–41)	10/29	25 (9.6–40)	5/16	33 (4.6–61)
NT4/5	30/30	299 (245–353)	45/45	291 (237–344)	29/29	276 (215–338)	16/16	318 (207–428)
GDNF	25/30	29 (21–37)	38/45	35 (27–42)	24/29	34 (23–44)	14/16	37 (25–49)

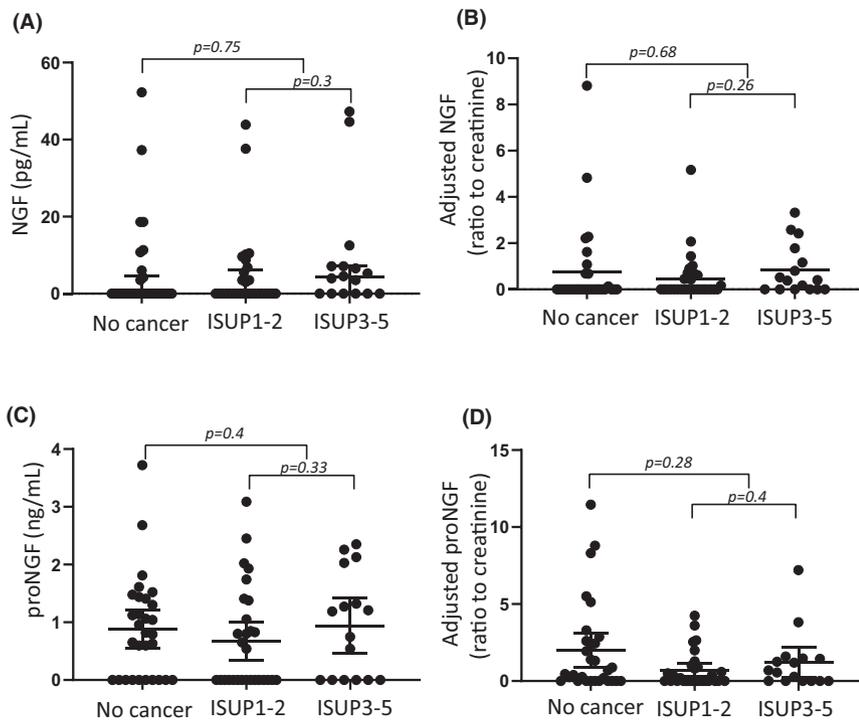


FIGURE 2 NGF and proNGF quantification in urine from cancer patients. NGF (A, B) and proNGF (C, D) quantification obtained by ELISA in urine from non-cancer subjects versus cancer patients with low-grade (ISUP1-2) and high-grade (ISUP3-5) prostate cancer. NGF and proNGF concentrations are presented in pg/ml (A, C) and after normalization with creatinine (B, D). ISUP, International Society of Urological Pathology

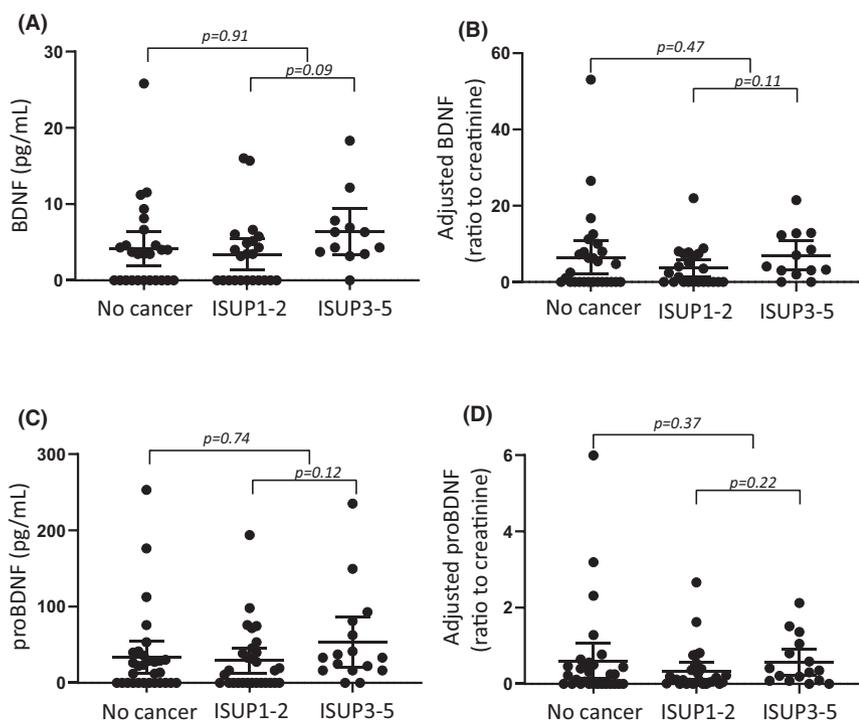


FIGURE 3 BDNF and proBDNF quantification in urine from cancer patients. BDNF (A, B) and proBDNF (C, D) quantification obtained by ELISA in urine from non-cancer subjects versus cancer patients with low-grade (ISUP1-2) and high-grade (ISUP3-5) prostate cancer. BDNF and proBDNF concentrations are presented in pg/ml (A, C) and after normalization with creatinine (B, D). ISUP, International Society of Urological Pathology

3.2 | Quantification of BDNF and proBDNF in urine

BDNF and proBDNF concentrations in urine samples from cancer patients versus non-cancer subjects are presented in Table 2 and Figure 3.

BDNF was detected in 23/36 (64%) of urine samples from cancer patients and in 15/26 (57%) of urine samples

from non-cancer subjects (Table 2), and the mean BDNF concentration was 4.3 pg/ml (95% CI, 2.7–5.9) in cancer urine samples versus 4.2 pg/ml (95% CI, 1.9–6.5) in non-cancer urine samples. When considering prostate cancer grades, 12/23 (52%) of ISUP1-2 urine samples and 1/12 (8%) of ISUP3-5 were positive for BDNF. The mean BDNF concentration was 3.4 pg/ml (95% CI, 1.4–5.4) in ISUP1-2 versus 6.4 pg/ml (95% CI, 3.4–9.5) in ISUP3-5. There was

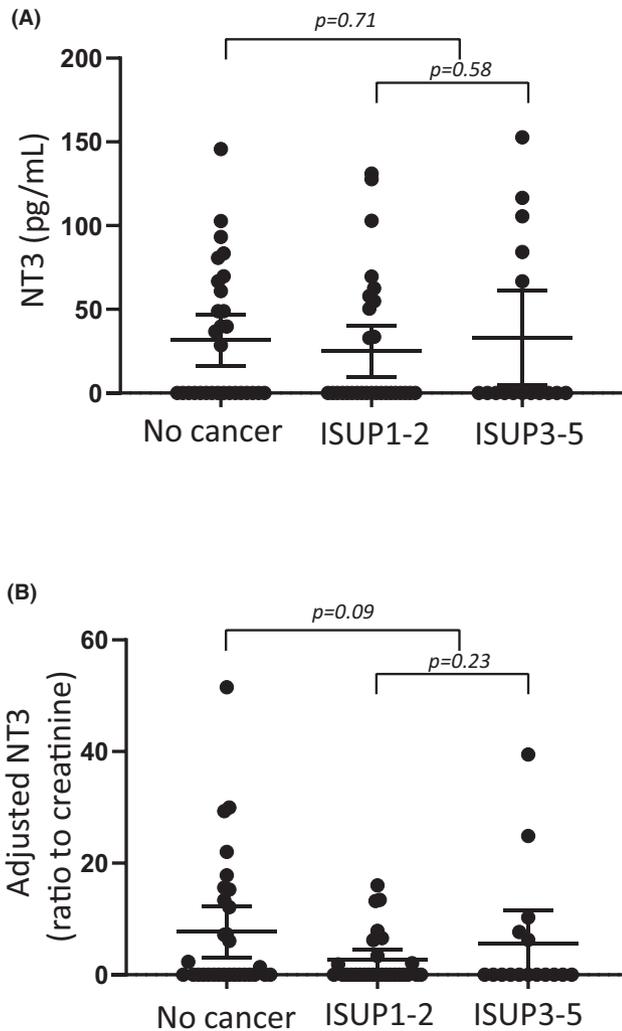


FIGURE 4 NT-3 quantification in urine from cancer patients. NT-3 quantification obtained by ELISA in urine from non-cancer subjects versus cancer patients with low-grade (ISUP1-2) and high-grade (ISUP3-5) prostate cancer. NT-3 concentrations are presented in pg/ml (A) and after normalization with creatinine (B). ISUP, International Society of Urological Pathology

no significant difference in BDNF concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 3A). Normalization of BDNF concentration to creatinine exhibited the same distribution with no significant difference between groups (Figure 3B).

ProBDNF was detected in 30/45 (66%) of urine samples from cancer patients and 18/30 (60%) of urine samples from non-cancer subjects (Table 2), and the mean proBDNF concentration was 38 pg/ml (95% CI, 22–53) in cancer urine samples versus 34 pg/ml (95% CI, 13–55) in non-cancer urine samples. When considering prostate cancer grades, 16/29 (55%) of ISUP1-2 urine samples and 14/16 (87%) of ISUP3-5 were positive for proBDNF. The mean proBDNF concentration was 29 pg/ml (95% CI,

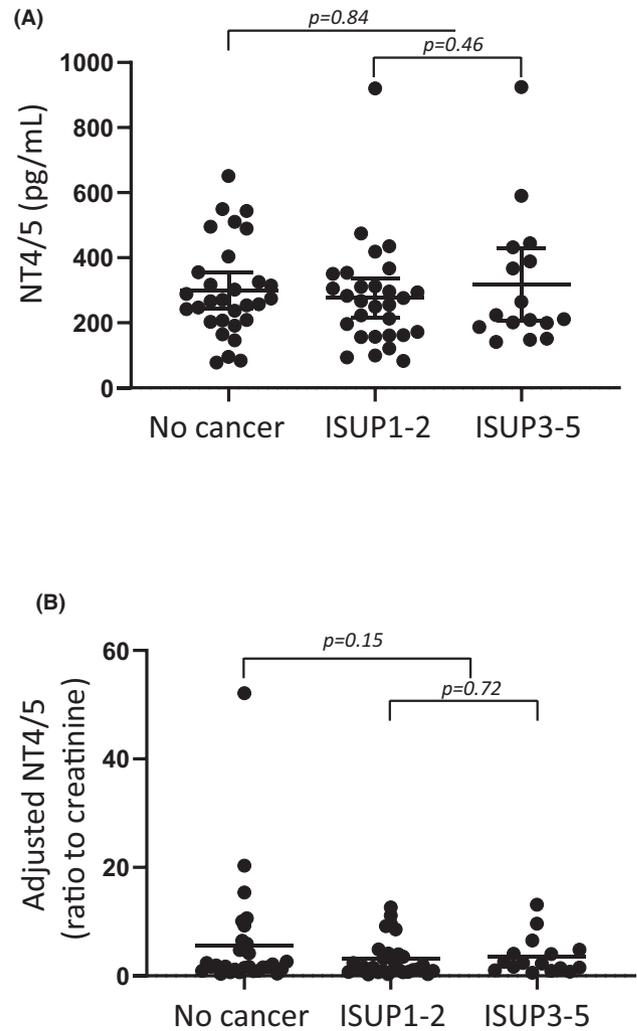


FIGURE 5 NT-4/5 quantification in urine from cancer patients. NT-4/5 quantification obtained by ELISA in urine from non-cancer subjects versus cancer patients with low-grade (ISUP1-2) and high-grade (ISUP3-5) prostate cancer. NT-4/5 concentrations are presented in pg/ml (A) and after normalization with creatinine (B). ISUP, International Society of Urological Pathology

13–45) in ISUP1-2 versus 54 pg/ml (95% CI, 21–87) in ISUP3-5. There was no significant difference in proBDNF concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 3C). Normalization of proBDNF concentration to creatinine exhibited the same distribution with no significant difference between groups (Figure 3D).

3.3 | Quantification of NT-3 in urine

NT-3 concentrations in urine samples from cancer patients versus non-cancer subjects are presented in Table 2 and Figure 4. NT-3 was detected in 15/45 (33%) of urine

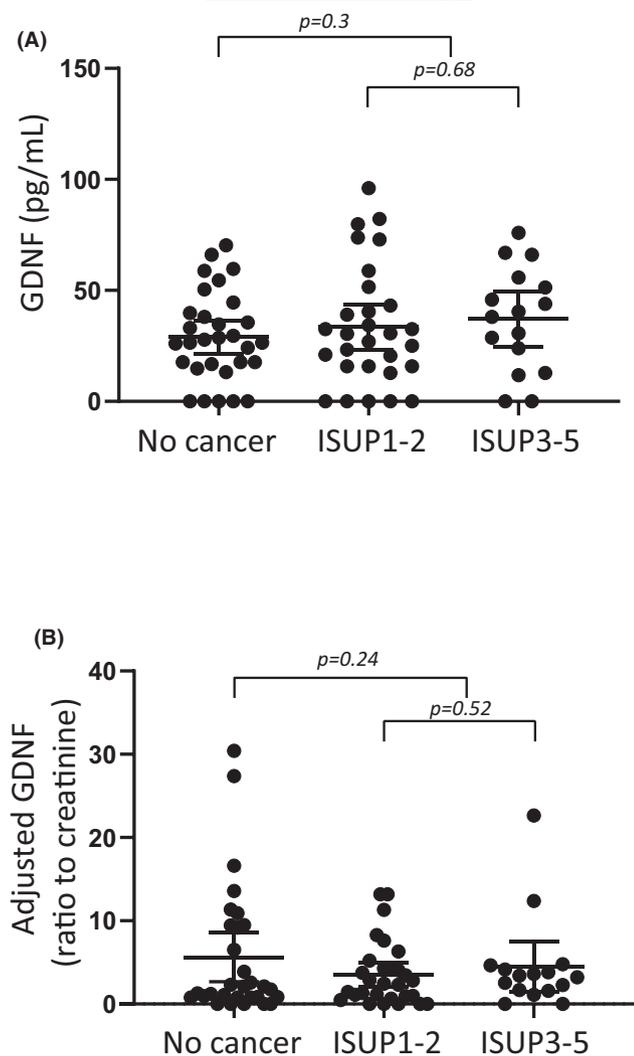


FIGURE 6 GDNF quantification in urine from cancer patients. GDNF quantification obtained by ELISA in urine from non-cancer subjects versus cancer patients with low-grade (ISUP1-2) and high-grade (ISUP3-5) prostate cancer. GDNF concentrations are presented in pg/ml (A) and after normalization with creatinine (B). ISUP, International Society of Urological Pathology

samples from cancer patients and in 14/30 (46%) of urine samples from non-cancer subjects (Table 2), and the mean NT-3 concentration was 27.8 pg/ml (95% CI, 14–41) in cancer urine samples versus 32 pg/ml (95% CI, 16–47) in non-cancer urine samples. When considering prostate cancer grades, 10/29 (34%) of ISUP1-2 urine samples and 5/16 (31%) of ISUP3-5 were positive for NT-3. The mean NT-3 concentration was 25 pg/ml (95% CI, 9.6–40) in ISUP1-2 versus 33 pg/ml (95% CI, 4.6–61) in ISUP3-5 cancers. There was no significant difference in NT-3 concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 4A). Normalization of NT-3 concentration to creatinine exhibited the same distribution with no significant difference between groups (Figure 4B).

3.4 | Quantification of NT-4/5 in urine

NT-4/5 concentrations in urine samples from cancer patients versus non-cancer subjects are presented in Table 2 and Figure 5. NT-4/5 was detected in all urine samples from cancer patients and non-cancer subjects (Table 2), and the mean NT-4/5 concentration was 291 pg/ml (95% CI, 237–344) in cancer urine samples versus 299 pg/ml (95% CI, 245–353) in non-cancer urine samples. When considering prostate cancer grades, all ISUP1-2 and ISUP4-5 urine samples were positive for NT-4/5. The mean NT-4/5 concentration was 276 pg/ml (95% CI, 215–338) in ISUP1-2 versus 318 pg/ml (95% CI, 207–428) in ISUP3-5 cancers. There was no significant difference in NT-4/5 concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 5A). Normalization of NT-4/5 concentration to creatinine exhibited the same distribution with no significant difference between groups (Figure 5B).

3.5 | Quantification of GDNF in urine

GDNF concentrations in urine samples from cancer patients versus non-cancer subjects are presented in Table 2 and Figure 6. GDNF was detected in 38/45 (84%) of urine samples from cancer patients and in 25/30 (83%) of urine samples from non-cancer subjects (Table 2), and the mean GDNF concentration was 35 pg/ml (95% CI, 27–42) in cancer urine samples versus 29 pg/ml (95% CI, 21–37) in non-cancer urine samples. When considering prostate cancer grades, 24/29 (82%) of ISUP1-2 urine samples and 14/16 (87%) of ISUP3-5 were positive for GDNF. The mean GDNF concentration was 34 pg/ml (95% CI, 23–44) in ISUP1-2 versus 37 pg/ml (95% CI, 25–49) in ISUP3-5 cancers. There was no significant difference in GDNF concentration between cancer versus non-cancer urine samples or between cancer of different grades (Figure 6A). Normalization of GDNF concentration to creatinine exhibited the same distribution with no significant difference between groups (Figure 6B).

4 | DISCUSSION

Compared with other biofluids such as serum, urine has many advantages for biomarker research. It is simple and non-invasive to collect, available in large quantities, and harmless to the human body. Furthermore, there is no significant proteolytic degradation in urine and it has a less complex matrix composition compared with serum or plasma, thereby reducing interferences and creating the potential for new biomarker discovery.²⁶ Despite the

advantages of urinary proteomics, and a number of potential protein biomarkers being proposed for the detection of prostate cancer, these candidates have not been translated to clinical practice.²⁷

In this study, we show that all neurotrophins and GDNF can be detected by ELISA in human urine, with no significant differences observed between urine of prostate cancer patients versus non-cancer subjects or between prostate cancer patients of different grades. In about half of all urine samples, these neurotrophic factors were not detected and the source of neurotrophic factors in the positive samples is to be determined. The prostate is a known source of neurotrophic factors^{17,18,20,21} and our hypothesis was that we could therefore detect neurotrophic factors in urine of prostate cancer patients, but it cannot be excluded that other organs or tissues could also release neurotrophic factors in urine. For instance, bladder cells have been shown to produce neurotrophins, including NGF, BDNF, and NT-3,^{28,29} which are increased in bladder dysfunction. Therefore, bladder-derived neurotrophic growth factors could contribute to the detection of neurotrophic factors in urine. Further investigations to determine the origin of the neurotrophic growth factors detected in urine are warranted.

According to our ELISA-based detection, the most abundant neurotrophic factor in urine is proNGF, with concentrations ranging between 0 and 1400 pg/ml, followed by NT-4/5 with concentrations between 207 and 428 pg/ml. In contrast, NT-3 (0–61 pg/ml) and proBDNF (0–87 pg/ml) were generally less abundant, and NGF (0–17 pg/ml) and BDNF (0–9.5 pg/ml) were found at even lower concentrations. It is important to note that all concentrations were above the limits of detection. The fact that proNGF seems to be the most abundant neurotrophic factor in urine suggest a particular importance for this neurotrophic factor in the urinogenital tract. Interestingly, proNGF has already been shown to be overexpressed in prostate cancer¹⁷ where it is associated with tumor grade and neural infiltration. *In vitro*, proNGF released by prostate cancer cells can stimulate neurite outgrowth and therefore it is thought that the release of proNGF by prostate cancer cells participate in the innervation of the tumor microenvironment.¹⁷ NGF has already been reported in human urine from prostate cancer patients¹⁹ at similar concentration range to what we have found here. This last publication also suggested that higher-grade tumors would be associated with more NGF in the urine, but we have not confirmed this in our present study and NGF was found at equivalent levels between cancer and control urine samples. In any case, our study reveals that when proNGF is present in urine, its concentration is about 10 times higher than NGF

concentrations. It has previously been shown that in the CNS proNGF is the major protein product of the NGF gene expression with a proNGF/NGF ratio is largely in favor of proNGF³⁰ and we confirmed the same quantitative predominance of proNGF versus NGF in the urine.

For each of the neurotrophic growth factors investigated in this study, there were no significant differences in concentration observed between urine from prostate cancer versus non-cancer men. Therefore, neurotrophic factors in urine do not appear to be biological markers for prostate cancer diagnosis. Similarly, there was no significant differences in neurotrophic factor concentrations between low-grade (ISUP1-2) and high-grade (ISUP3-5) cancers, indicating that neurotrophic factors are unlikely to be prognostic biomarkers in prostate cancer. However, and this is a limitation of our study, the size of the cohort used here is limited (45 cancers vs. 30 control men) and could account for the lack of significant differences between groups. Therefore, now that we have established the presence of neurotrophic growth factors in the urine of prostate cancer patients, further clinical studies involving larger size cohorts are needed to clarify the potential clinical utility of neurotrophic growth factors as biomarkers in prostate cancer. Another limitation of our study is the sole use of ELISA to assay neurotrophic growth factors. Although the ELISA test that we have used are robust and reliable, the detection and quantification of neurotrophic factors that we report would need to be confirmed by alternative methodologies, such as Western blotting and mass spectrometry. These alternative approaches would also provide more information about an eventual processing of neurotrophic growth factors in the urine.

Overall, this study indicates that neurotrophic growth factors can be quantified in urine by ELISA. Although we have not found any difference in neurotrophic growth factor concentration between cancer patients versus control men or between patients with different tumor grades, the presence of neurotrophic factors in this pilot study, further investigations on larger patient cohorts are warranted to test the potential utility of neurotrophic growth factors as clinical biomarkers for cancers of the urogenital tract.

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CONFLICT OF INTEREST

The authors declare no competing interest with the content of this manuscript.

AUTHORS' CONTRIBUTION

BM and HH have designed the study. SF participated in ELISA and data analysis. XY has been involved in the collection of clinical and biological samples. BM and HH have drafted the manuscript. The final manuscript has been approved by all co-authors.

ORCID

Sam Faulkner  <https://orcid.org/0000-0002-4732-8252>

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